An Exciting Release on TRPM7

Following fusion, synaptic vesicles do not always release all of their neurotransmitter. According to one model, neurotransmitters bind to a charged matrix within secretory vesicles, and release requires entry of counterions. In the current issue of *Neuron*, Krapivinsky et al. demonstrate that TRPM7 is localized to synaptic vesicles and is required for release of the positively charged neurotransmitter acetylcholine. The results raise the possibility that TRPM7 is the enigmatic channel that supplies counterions for neurotransmitter release.

Excitement concerning the roles of TRP channels in excitable cells is already high. After all, these channels are well known to play critical roles in sensory modalities, such as touch, hearing, taste, olfaction, and vision, in animals ranging from worms to flies, mice, and humans. Of particular note, multiple TRP channels act as thermosensors and participate in nociception. During the last few years, it has become clear that the functions of TRPs in the nervous system extend well beyond contributions to the Aristotelian senses to include roles in neurite outgrowth, growth cone guidance, and even synaptic transmission. For example, in thalamic interneurons isolated from TRPC4 knockout mice, the release of the neurotransmitter GABA is reduced drastically (Munsch et al., 2003).

Insight into the mechanism by which TRP channels facilitate synaptic transmission is provided in an article by Krapivinsky et al. in the current issue of *Neuron* (Krapivinsky et al., 2006). These authors provide compelling evidence that TRPM7 is situated in membranes of synaptic vesicles and that this localization is necessary for the normal release of the neurotransmitter acetylcholine from sympathetic neurons. An important finding is that TRPM7 does not appear to affect the number of vesicles that fuse with the presynaptic terminal. Rather, reductions in TRPM7 expression diminish quantal size—that is, the amount of neurotransmitter released following fusion of individual vesicles with the plasma membrane. Most importantly, the mechanism through which TRPM7 is suggested to regulate the extent of release is fascinating, and is described below.

The study by Krapivinsky et al. did not begin with a preconceived notion that TRPM7 functioned in neurotransmission. Rather, in a quest for clues as to the normal function of this channel, the authors performed a yeast two-hybrid screen for TRPM7 interacting proteins. The basis for the interest in TRPM7, and the highly related TRPM6, is that these channels have a number of unusual features that distinguish them from other TRPs. These include relatively high conductivity to Mg^{2+} and an atypical protein kinase domain linked at the C terminus to the channel domain.

Among the positives isolated in the yeast two-hybrid screen was snapin, a synaptic vesicle protein that binds to a SNARE protein, SNAP-25, that contributes to the fusion of synaptic vesicles with the presynaptic plasma membrane. The snapin/SNARE interaction in turn augments binding of synaptotagmin, which promotes synaptic transmission. In support of the initial yeast two-hybrid studies, Krapivinsky and colleagues find that TRPM7 is present in synaptic vesicle preparations in a complex with snapin, synaptotagmin, and another synaptic vesicle protein, synapsin. Furthermore, TRPM7 was localized to presynaptic vesicles at the neuromuscular junction and in superior cervical ganglion (SCG) neurons, which contain the positively charged neurotransmitter acetylcholine.

A key question is whether TRPM7 functions in synaptic vesicles or is silently stored in these vesicles, waiting to be translocated to the plasma membrane. Most TRP channels are thought to function exclusively in the plasma membrane, where they promote cation influx. However, TRPs are also detected in intracellular vesicles, and in some cases, TRP-dependent cation influx is activated through regulated translocation to the plasma membrane. Two examples of particular relevance to the new study on TRPM7 are the demonstrations that TRPC3 and TRPV1 bind to the SNARE protein VAMP2 (also known as synaptobrevin), and snapin and synaptotagmin, respectively; these interactions appear to augment exocytotic insertion of the channels in the plasma membrane (Morenilla-Palao et al., 2004; Singh et al., 2004).

The central result in the report by Krapivinsky et al. is that TRPM7 is required for synaptic transmission, rather than required to facilitate cation influx at the plasma membrane of the presynaptic terminals. TRPM7 was detected exclusively in preparations of synaptic vesicles and in a complex with synaptic vesicle proteins; moreover, suppression or elevation of the levels of TRPM7 had corresponding effects on release of the neurotransmitter. To decrease or increase TRPM7 expression, the authors injected either TRPM7 siRNAs or a vector encoding TRPM7 into the presynaptic neurons from rat SCG. Krapivinsky et al. then induced action potentials in the presynaptic neurons and assayed excitatory postsynaptic potentials (EPSPs) from the postsynaptic neurons as a measure of neurotransmitter release. The amplitudes, quantal sizes, and decay times of the EPSPs changed in parallel with the levels of TRPM7 expression. Disruption of the TRPM7-snapin interaction, through perfusion of a truncated snapin or TRPM7 peptide, had a similar effect to knockdown of TRPM7 expression. However, the number of vesicles that released neurotransmitter did not appear to be affected. The requirement for TRPM7 reflected a role for cation conductance, rather than some other function of TRPM7, since a dominant-negative mutation that suppressed the TRPM7 current also inhibited synaptic transmission.

So, why is TRPM7 needed for normal release of acetylcholine? Release of neurotransmitters from synaptic vesicles is not dependent simply on fusion of the synaptic vesicles with presynaptic membrane—that is, release

is not an all or nothing event in which the contents of a synaptic vesicle are completely emptied following fusion. An intriguing proposal for TRPM7 function put forth by the authors builds on earlier work suggesting that charged neurotransmitters are not free and mobile in the vesicles, but associate with an "ion exchange matrix," possibly comprised of proteoglycans (Rahamimoff and Fernandez, 1997; Reigada et al., 2003) (Figure 1A). Release from the matrix requires entry of a counterion into the vesicles, which would displace the neurotransmitter from the matrix (Figure 1B). Since acetylcholine is positively charged, the matrix would be negatively charged and the counterion would be a positively charged species, such as Na^+ or Ca^{2+}.

One of the major questions raised by the "ion exchange hypothesis" is the identity of the ion channel that permits the flow of cations into the vesicles. Could TRPM7 be such an ion exchange channel? Consistent with this possibility, the TRPM7 cation channel is localized to synaptic vesicles and a reduction in the kinetics and size of the release results from knockdown of TRPM7 expression or disruption of the TRPM7-snapin interaction. If TRPM7 promotes cation uptake into the intralumenal space, in view of the presumed cytoplasmic topology of the N and C termini, then the direction of cation flow would be opposite to that of TRPM7 channels in the plasma membrane. The concept that TRPM7 promotes cation influx into the lumen of the synaptic vesicles raises many remaining questions, including the basis for the requirement for TRPM7 to bind to snapin to promote neurotransmitter release. The cations flowing into the vesicles are not known; however, it is unlikely to be Na^+, since TRPM7 is primarily permeant to divalent cations, such as Ca^{2+} and Mg^{2+} (Nadler et al., 2001). Since the free concentrations of cations in the synaptic vesicles are not well defined, it is unclear if there is sufficient driving force to promote the flow of cations into the synaptic vesicles. Nevertheless, Ca^{2+} levels can rise to high levels in the presynaptic cleft, potentially providing the necessary driving force.

Another question is whether TRPM7 functions prior to the fusion event or postfusion. In support of the original ion exchange hypothesis (Rahamimoff and Fernandez, 1997), Krapivinsky et al. argue in favor of activation of TRPM7 occurring either following attachment or fusion of the vesicles to the plasma membrane. The basis for this proposal is that PIP_2 is needed for TRPM7 activation, but is present only in the plasma membrane, and not in the membranes of synaptic vesicles (Runnels et al., 2002) (Figure 1B). However, since activation of TRPM7 is also promoted by low pH (Jiang et al., 2005), the channel may be rapidly inactivated following fusion, since this event would neutralize the low pH in the vesicle lumen (Krapivinsky et al., 2006) (Figure 1C).

The concept that TRPM7 provides the counterions for release of acetylcholine has considerable appeal; however, one can envision other possibilities to account for TRPM7's contribution toward release. Converse to the concept that TRPM7 facilitates entry of cations into synaptic vesicles, TRPM7 could release cations from the vesicles into the cytoplasm. Such a phenomenon might enhance uptake of acetylcholine into vesicles, which in turn would affect quantal size. According to the "kiss and run" model of exocytosis, fusion of synaptic vesicles with the plasma membrane generates a transient fusion pore. Thus, another possibility is that TRPM7 activity is necessary for full dilation of the fusion pore, although some studies indicate that the size of the pore is not the factor limiting release. An important future experiment to contribute to our understanding of the role of TRPM7 in neurotransmitter release would involve

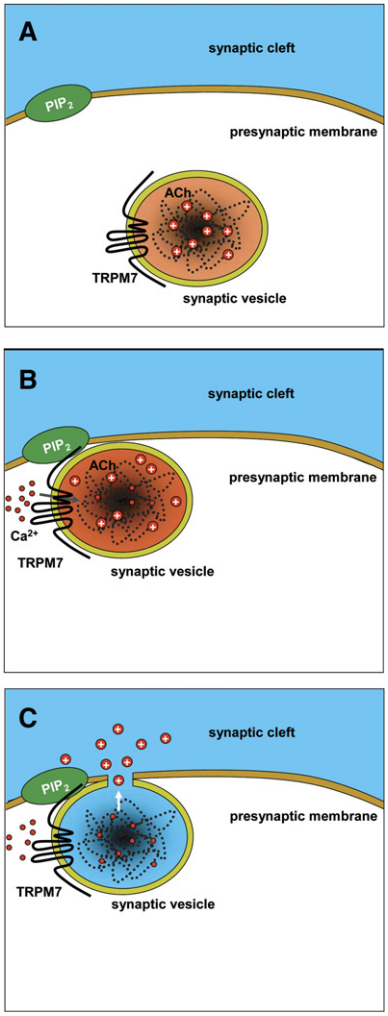

Figure 1. Ion Exchange Model

(A) Binding of acetylcholine to an ion exchange matrix in the synaptic vesicles. Prior to attachment and fusion, the positively charged acetylcholine (ACh; indicated by "+") is bound to a negatively charged ion exchange matrix. The acidic pH in the lumen of the synaptic vesicle is indicated by the orange shading. PIP_2 is present in the plasma membrane, but not the membrane of the synaptic vesicle.

(B) Displacement of ACh from the ion exchange matrix. Following attachment of the vesicles to the plasma membrane, TRPM7 is activated by the combination of low pH and PIP_2. The influx of divalent cations (e.g., Ca^{2+}) leads to displacement of ACh from the negatively charged matrix.

(C) Release of ACh from the synaptic vesicle. According to the kiss and run model, fusion of secretory vesicles with the plasma membrane leads to formation of transient fusion pores. The displaced ACh is released from the synaptic vesicle and the acidic pH in the lumen of the synaptic vesicle is dissipated, thereby terminating activity of TRPM7.

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analyzing the biophysical properties of the TRPM7 conductance by performing patch-clamp studies on fused giant synaptic vesicles. Such studies would also facilitate characterization of the mechanism through which TRPM7 may be activated in the membrane of synaptic vesicles. TRPM7 is a "chanzyme" consisting of fused channel and protein kinase domains (Nadler et al., 2001; Runnels et al., 2001), and while the authors showed that the channel activity is clearly required for neurotransmitter release, we do not know if the protein kinase contributes to TRPM7 function in synaptic vesicles.

Despite questions concerning the exact mechanism through which TRPM7 regulates release of acetylcholine, the current study provides the first demonstration of a role for a Group 1 TRP in an intracellular membrane, as opposed to the plasma membrane. The Group 1 TRPs include TRPs that fall into the five subfamilies (TRPC, TRPV, TRPM, TRPA, and TRPN) that are most related to the original member of the superfamily, *Drosophila* TRP. Members of the distantly related Group 2 TRPs, such as TRPP2 and TRPML1, are known to be present primarily in intracellular compartments, although their precise functions remain elusive. The concept that such TRP channels are waiting in a dormant state to undergo regulated translocation to the plasma membrane almost certainly applies in some cases. Nevertheless, given the findings by Krapivinsky et al., it may turn out that many TRP channels, which are currently considered cation influx channels, have equally important roles in a variety of secretory vesicles and intracellular organelles.

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Can One Suppress Subliminal Words?

Subliminal words cause behavioral priming, yet the depth of their processing remains debated. Using transcranial magnetic stimulation (TMS), Nakamura et al. demonstrate in this issue of *Neuron* that this subliminal priming effect can be selectively disrupted. Distinct TMS sites disrupt priming in lexical decision and pronunciations tasks, suggesting that task set influences subliminal processing.

The topic of subliminal images readily evokes an infamous episode of the US presidential campaign where a republican television clip associated Al Gore's face with the subliminal presentation of the word "rats." Psychologists have long known that words that are briefly flashed can easily be made invisible by preceding and following them with nonsense shapes that serve as visual masks. The key issue, which has been stimulating intense experimental research since Tony Marcel's seminal studies in the 1980s, is how deeply are such subliminal words processed. Can they activate orthographic, phonological, or even semantic levels of representation? Which brain areas do they contact? Can the conscious strategies adopted by the subjects shape the path that they take? In this issue of *Neuron*, Nakamura et al. (2006) provide an elegant answer to some of these questions.

The authors asked Japanese subjects to perform either a lexical decision task or a pronunciation task on words and pronounceable nonwords that were presented visually or auditorily. Unbeknownst to the subjects, a subliminal visual word, which could be identical or distinct from the target word, was also presented on each trial. In a first experiment, Nakamura and his colleagues show that this hidden word produces reliable repetition priming effects. In both tasks, subjects responded faster to repeated stimuli than to nonrepeated prime-target pairs. Remarkably, this subliminal priming effect was observed within the visual modality, but also crossmodally (from a visual prime to an auditory target).

In a second experiment, the authors replicated this experiment while single-pulse transcranial magnetic stimulation (TMS) was applied either to a left inferior parietal area previously thought to play a role in grapheme-phoneme conversion or to a left superior temporal area implicated in crossmodal word integration. The original aspect of their study was to examine whether TMS, which was applied just prior to the prime presentation, could suppress subliminal repetition priming. The answer, remarkably, depended on the task. When subjects were consciously engaged in the lexical decision task, TMS of temporal cortex abolished behavioral repetition effects, while parietal TMS left repetition priming intact. Conversely, when subjects were engaged in the pronunciation task, parietal TMS but not temporal TMS suppressed repetition priming. This double dissociation strongly suggests that the very same masked words were processed through distinct neural pathways depending on the task performed. Curiously enough, target-driven response times were not affected by TMS,